

## The cysteine protease TcCYSPR04 *T. cacao* accumulates in senescent leaves and change the biotrophic phase for saprophytic tissues infected by *M. perniciosa*

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A cysteine proteinase named *TcCYSPR04* was identified in a cDNA library of the *Theobroma cacao*–*Moniliophthora perniciosa* interaction, in the EST<sub>tik</sub>-CIRAD database and in the cacao genome of MARS. *TcCYSPR04* presents an ORF of 1068 bp encoding protein with: (i) a molecular weight and isoelectric point of 39 kDa and 5;43, respectively; (ii) a signal peptide with a probable cleavage site between the amino acids 19 and 20; (iii) an inhibitory domain between amino acids 56 and 112; and (iv) a catalytic domain between amino acids 158 and 353. *TcCYSPR04* may be secreted or cytoplasmic. According to the literature, the cysteine proteases may be involved in cell differentiation, senescence, and programmed cell death-PCD. The catalytic domain is highly conserved among cysteine proteases and was subcloned into pET28a expression vector. The corresponding protein was expressed in strain *Escherichia coli* BL21 (DE3) and purified by affinity column His-Trap. Polyclonal antibodies against the recombinant protein were produced in rabbits and purified by immunoadsorption. Total proteins were extracted from apoplastic fluid of healthy and infected leaves of resistant and susceptible varieties of cacao. Proteins were subjected to electrophoresis on SDS-PAGE 15%, and immunoblot using the serum anti-*TcCYSPR04*.: *TcCYSPR04* was immunodetected in senescent leaves infected by *M. perniciosa* at different development stages and in apoplastic fluid. According to these results, *TcCYSPR04* may participate in plant senescence, cell death and defense in response to pathogen attack.

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